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RESEARCH ARTICLE

CHEMICAL CHARACTERIZATION AND ANTI-DISEASE PROPERTIES OF POULTRY COMPOST AND CHILLI EXTRACT AGAINST TOMATO FOLIAR AND FRUITS DAMAGES

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ABSTRACT

This study was undertaken to formulate a biopesticide based poultry compost tea and aqueous chilli extract for the evaluation of their efficacy on tomato foliar and fruit damages in the field. A completely randomised block design was set up, consisting of five treatments triplicated each, and including a negative control and four insecticide treatments (compost tea, aqueous chilli extract, compost tea+aqueous chilli extract and Decis). Plants were linearly transplanted in the field using a string, with only plants carrying at least 5 to 6 leaves sown singly to the level of the first leaves, at 40 cm x 40 cm apart within and between lines. As from the 14 days after transplantation plants were sprayed, then parameters such as size at different flowering dates, number and weight of fruits/plant were recorded, whereas the foliar and fruits damages were observed. Damages observed in the field were fruits rot (anthracnose) and leaf discoloration (chlorosis). The formulated biopesticides (poultry compost tea-chilli extract) were found to have a significant effect ($p = 0.0001$) on plants size, with 2 folds increased over the negative control. The average number of fruits/treatments varied significantly ($p = 0.0004$), the highest values accounting for treatments compost tea-aqueous chilli extract (T3) and the pesticide mixture (T5), which contributed to 2 to 2.5 folds increased fruit yield in the field. These pioneer results open up prospects for the use of biopesticides based compost tea and chilli extract to combat tomato foliar and fruits damages in the field.

KEYWORDS

anthracnose, chlorosis, tomato, chicken manure compost tea, aqueous chilli extract

1. INTRODUCTION

African population is expected to reach 2.5 billion by 2050, representing the largest demographic surge in the world (United Nation projections, 2019). This population growth has resulted in 12% of the world's population being undernourished, creating a mismatch between population growth and fresh fruit and vegetable production in developing countries (Droh et al., 2022). This has sparked particular interest among farmers, of the market gardening sector especially for tomatoes, due to their place in the household basket (Kouakou et al., 2010). Scientifically known as *Solanum lycopersicum* (L.), tomato is an herbaceous plant of the Solanaceae family that is highly valued for its nutritional content. Tomato fruits are rich in minerals, vitamins, amino acids, sugars and fibre (Agrodok, 2005). Its relatively short life cycle and high yield also make it a crop of great economic interest (Agrodok, 2005). Despite its importance, production remains low due to various constraints faced by farmers, such as diseases, pests and poor soils. To tackle these constraints, growers regularly use chemical inputs known as pesticides, which are used to control or repel pests and disease vectors in order to improve crop yields (INRA, 2005). However, these chemicals cause real threats to the environment and human health, including increased cancer risks, soil and groundwater contamination, reduced biodiversity and toxicity to non-target organisms such as pollinating insects (Le Bars et al., 2020). In order to quantitatively and qualitatively boost the production, it is essential to develop new eco-friendly environmental strategies. One of these proposed strategies is the use of aqueous chilli extract and chicken manure compost tea. Chilli (*Capsicum annuum*, Solanaceae), is not only a condiment, but is also a bioinsecticide widely used in horticulture due to its diversity of active compounds, including capsaicinoids and polyphenols (Ngando et al., 2022). Compost tea on its own, is very rich in beneficial microorganisms that can act as biological control agents against pests, pathogens, but is also known to strengthen the immune system of plants (Tóthné et al., 2021). The present study was carried out to search for alternatives to chemical control; through evaluation of the biological efficacy of aqueous

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chilli extract combined with chicken manure compost tea on tomato foliar and fruits damages, in a view to preserve the environment and alleviate costs associated with the phytosanitary protection of this Solanaceae. In this research we produced and characterized chicken manure compost tea, then evaluated their combined and separate effects on tomato growth, yield and protection against damages.

2. Materials And Methods

2.1. Description of the study site and biological materials

The experiment was carried out in the Guinean Savannah agro-ecological zone at Dang, within the experimental field of the Unit for Apply Apidology (latitude: 7°25.119 N ; longitude : 13°33.415 E ; altitude : 1106 m a.s.l.) of the Faculty of Science, University of Ngaoundere, Cameroon. The climate is characterized by a rainy season (April to October) and a dry season (November to March), with an annual rainfall of approximately 1500 mm. The mean annual temperature is 22°C, while the mean annual relative humidity is 70 % (Amougou et al., 2015). The biological material represented by *Solanum lycopersicum* seeds of the Rio Grande variety (Figure 1) was provided by the Institute of Agricultural Research and Development (IRAD) of Wakwa-Ngaoundere, while the composting substrate used was 300 kg chicken droppings collected from a known chicken breeder (Figure 2).

Figure 1 : Chicken droppings

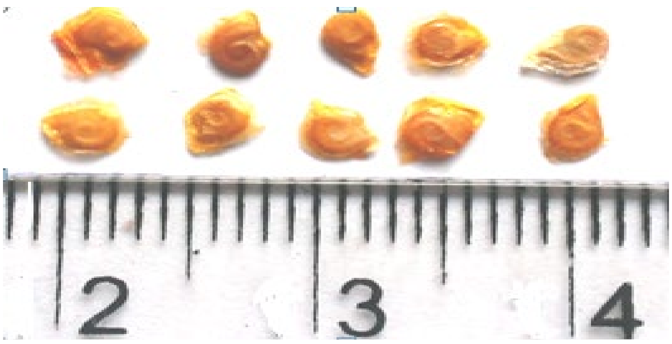

Figure 2 : *Solanum lycopersicum* seeds

2.2 Composting process and preparation of compost tea

The composting technic used to produce compost from chicken manure was the aerobic composting in piles according to the method of (Ngakou et al., 2008 ; Gnimassoun et al., 2020). The compost piles were composed of alternate layers of straw, inoculum and manures that were monitored until all the waste is used up. After each layer, the compost heap was watered to prevent low moisture content. Once the heap was built up, holes were drilled with a strike for aeration and then covered with a white plastic paper. The composted chicken manure was dried in the shade for three weeks, then ground into powder through porcelain mortar and sieved.

Aerated compost tea (Figure 3A) was a compost extract infused in water. The aim was to extract and multiply the micro-organisms present in the compost into water, so that the resulting product can be sprayed on plants. Averagely 5 kg of mature compost powder was needed for 20 litres of water. The compost powder was added to an open container of water and stirred gently with a sterile spatula for 45 minutes to oxygenate the medium and ensure the proliferation of aerobic micro-organisms. The mixture was then left to ferment for 48 hours and filtered through a 50 µm mesh to recover the compost tea (Ngakou et al., 2014 ; Alium et al., 2021).

2.3 Chemical characterisation of Compost Teas

2.3.1. Determination of pH of samples

To determine the pH of samples, 100 g of each sample (chicken droppings powder and compost from chicken droppings) was taken and mixed with 500 mL of distilled water in a beaker. After stirring for 5 minutes, the mixture was left to stand for 2 hours. The pH of each solution was obtained by reading the value displayed by the pH meter when immersed the electrode in the sample (Aké et al., 2018).

2.3.2. Phytochemical tests

2.3.2.1. Alkaloids

Compost tea (1.0 mL) was dissolved in 2mL ethanol solution. After stirring with a glass rod in a water bath at 37°C, 2 to 3 drops of Dragendorff reagent were added. The appearance of a red or orange colour indicates the presence of alkaloids (Mengome et al., 2009).

2.3.2.2. Tanins

Compost tea (1 mL) was mixed with 2 drops of 1% FeCl3. The formation of a black, green or blue colour was the confirmation of a positive test (Gueye et al., 2022).

2.3.2.3. Flavonoids

Flavonoid determination was highlighted by the cyanidin reaction. Hence, 1 mL of compost tea was mixed with 5 mL of concentrated HCl in the presence of magnesium ion in test tubes. The appearance of a yellow, orange, red or purple colour indicated a positive test (El-haoud et al., 2018).

2.3.2.4. Saponosides

Compost tea (1 mL) was mixed with 4 mL of distilled water, before the mixture was stirred for 15 seconds. The appearance of foam was an indication of a positive test (Ano et al., 2018).

2.3.2.5. Terpenes and sterols

Terpenes and sterols were identified through the Libermann-Buchard reaction. Acetic anhydride (1 mL) was mixed with 0.5 mL chloroform, 0.5 mL concentrated H_2SO_4 and 1 mL compost tea in a test tube. After shaking, the appearance of a purple or violet band that turns into blue and then green indicated a positive test (Kachkoul et al., 2018).

2.3.2.6. Anthocyanin

In a test tube, 1 mL of compost tea was mixed with 2 mL of HCl (2N) and 2 mL of ammonia. After shaking, the appearance of a violet-blue colour showed the presence of anthocyanins (Kallo et al., 2018).

2.3.2.7 Phenolic compounds

In a test tube, 1 mL of each extract was mixed with a few drops of 2% ferric chloride. The presence of phenolic compounds was indicated by the appearance of a blackish blue or green colour (Walid et al., 2016).

2.4 Preparation of aqueous chilli extract and chemical pesticides

The aqueous extract of *Capsicum annuum* was prepared from 3 kg of chilli provided by a chilli breeder that was sun-dried for a week. The dried material was then ground in an electric mixer to a powder that was sieved through a 50 µm mesh to obtain the extract according to the method described by (N'guessan et al., 2007). The method consisted of weighing 100 g of chilli powder, adding it to 1L distilled water, leaving it for maceration for 48 hours and then filtering it through a N°1 Whatman paper. The obtained aqueous chilli extract was diluted before use in the field to avoid burning of leaves. For instance, 0.5 litre of pure aqueous chilli extract was diluted to 50% by adding 0.5 litre of water, followed by a vigorous shaking of the mixture (Figure 3b). However, to formulate the biopesticide was formulated from the compost tea + aqueous chilli extract mixture (50%, v/v) in 1.5L plastic, then shaken vigorously, before dilution at 50% with distilled water (Figure 3c).

The synthetic chemical pesticide solution was prepared according to the recommendations of the phytopharmacist and the instructions on the leaflet. Therefore, 5g Penncozeb 720 WP, 3ml Optimal 20 SP were mixed in a bucket with 5 L of water, as they are often used by farmers to control anthracnose and chlorosis. The shaken solution was transferred to a hand sprayer to be applied onto tomato plants in the field (Figure 3d).

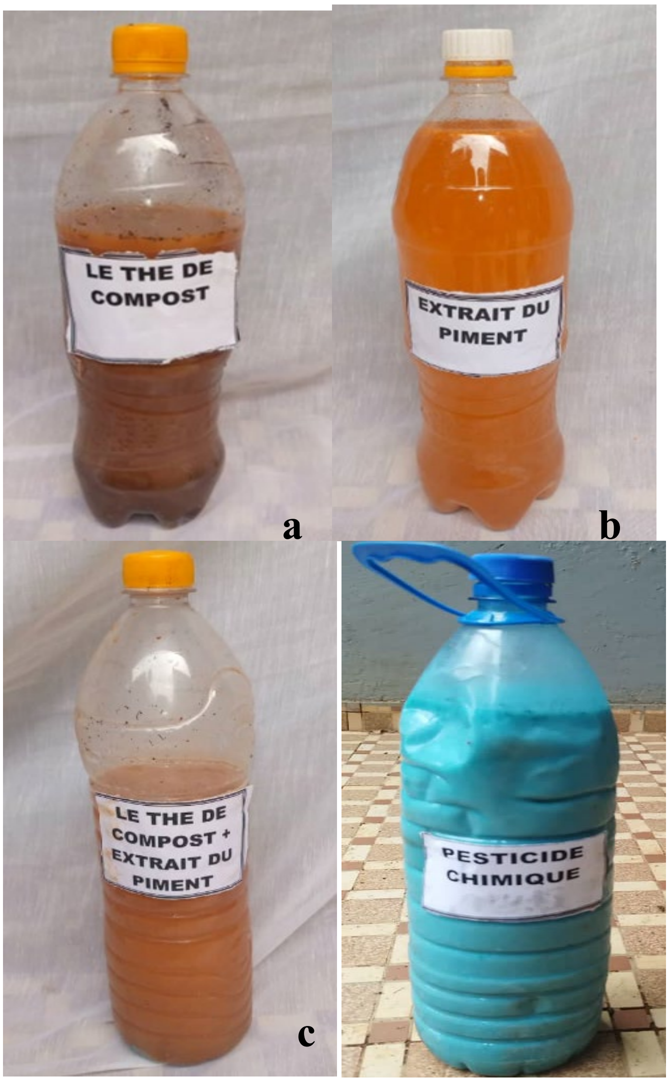

Figure 3 : Biological and chemical pesticides used: Compost tea (a); Aqueous chilli extract diluted to 50% (b); Compost tea + aqueous chilli extract (c); Chemical pesticides (d).

2.5 Plant material and experimental setup

From April 3 to July 29, 2023, a 324 m² experimental field was cleared, ploughed and divided into 15 plots, each measuring 2.5 m in length and 2.5 m in width, separated 1m apart to facilitate movement within the site during spraying. The experimental design was a completely randomised block consisting of five treatments, each of which was triplicated. Treatments were: aqueous chilli extract (T1); compost tea (T2); compost tea + aqueous chilli extract (T3); untreated plants or control (T4); pesticide mixture (T5). *Solanum lycopersicum* nursery was established by sowing the seeds in the soil, then watering and covering with straw to maintain humidity and facilitate seeds emergence. The nursery thus set up was watered in the morning and evening until transplantation after 45 days. Transplantation of 45 days old vigorous plants was performed in rows of five lines per experimental plot, with two plantlets per pocket, spacing 50 cm within and 70 cm between the lines, for a total of 25 plants per unit plot. Six weeks after transplantation, there was appearance of the first flowers, and the first fruits one month later. From flowering to fruit maturity, weeding was performed by hand. Finally, Insecticides were sprayed with 4 graduated manual sprayers, each sprayer corresponding to a single insecticide product. Treatments were applied by spraying plants in the morning between 06:00 and 08:00, taking into account the weather conditions that could affect the effectiveness of the spraying (Oparaeke, 2005). Plants were spread when the wind was low to avoid disorientation of the droplets of the product not reach their target (Omoigui et al., 2018). Treatments were applied once, 2 days apart, as soon as the flowers appeared.

2.6 Assessment of growth and yield parameters

The efficacy of the applied treatments was evaluated through the severity of disease symptoms of fruit rot; leaves chlorosis. Symptoms severity was recorded at the beginning of flowering (45 DAP) and at fruit maturity (75 DAP) for each plot on 1 to 3 scale (1 = low, 2 = medium, 3 = very severe) (Qaryouti et al., 2003; Adje et al., 2009). Growth and yield parameters included measurement of plant height with a graduated tape, number of flowers and number of ripe fruits per plant by direct counting, yield determination using the formula: R = (M x 31,250) / 1,000, where M is the weight of the fruit harvested from a plant, 31,250 is the number of tomato plants per hectare, and 1/1.000 is the conversion to tonnes (Egho and Emosairue, 2010).

2.7 Statistical Analysis

Data collected were ordered and classified within the Microsoft Excel 2013, transferred to Statgraphics Plus 16.0 software for analysis of variance (ANOVA). Pearson correlation tests were performed between the different parameters whereas means were separated between treatments using the (LSD) test.

3. Results And Discussion

3.1 Characteristics of different compost waste types

The pH of the different wastes varied from 8.31 to 8.34, corresponding to the alkaline pH range. These values are not close to those found pH ranging from 7.3 to 7.5 (Aké et al., 2018). According to this study, an alkaline pH indicates better control of the composting process, while an acidic pH indicates compost rich in acetic and lactic acids (Beck-Friis et al., 2001). Poultry manure has been reported to show a basic pH that favours the activity of actinomycetes and alkaline bacteria (Mustin, 1987). Phytochemical analysis was performed on aqueous extracts of composted and non-composted chicken manure to determine the presence or absence of secondary metabolites. Seven classes of metabolites were screened including phenolics, flavonoids, alkaloids, triterpenes, anthocyanins, saponins and tannins (Table 1).

Table 1: Metabolites in manure and compost extracts

Solvents	Extracts						
Water	Phenolic compounds	Flavonoids	Tanins	Saponins	Anthocyanins	Alkaloids (Meyer)	Triterpenes
A1	+	+	-	-	+	+	-
B1	+	+	-	-	+	+	-

(+): Presence; (-): Absence; A1: aqueous chicken droppings compost extract; B1: aqueous chicken droppings non-composted extract.

Table 1 indicates that phenolic compounds, flavonoids, alkaloids and anthocyanins were present in both extracts. Their presence in the extract was an indication of the activity of microorganisms involved in the degradation of the substrates. The absence of tannins, saponins and triterpenes in both extracts could be due either to the fact that water was not the appropriate solvent for their extraction, or the microorganisms able to synthesize these metabolites in the composted or non-composted chicken manures could have been destroyed during the extraction process (Lincoln et al., 2006 ; Al Naser, 2018).

3.2. Incidence of treatments on tomato growth and production parameters

3.2.1. Effect on plant size

No significant difference (p = 0.3616) was observed between the negative control (T4) and other treatments at 9 days after spraying, as far as plant size is concerned (Figure 4). However, at 37 days after spraying, treatments highly significantly differed (p = 0.0001) each other. Hence, aqueous chilli (T1) and compost tea (T2) extracts stimulated plant height growth than the negative control (T4), although the best height increase accounted to the combined aqueous extract treatments (T3), with an average of 36.47 cm per plant. This finding highlights the ability of the aqueous compost tea + chilli extracts mixture to enhance tomato plant height, confirming the presence of phenolic compounds in chilli extracts that protect tomato plants, ensuring better development, as well as the quality and quantity production (Ngando et al., 2022). These results are in line with those who revealed that *Lavandula officinalis*, *Thymus vulgaris*, *Cymbopogon citratus* and *Melissa officinalis* improved the agronomic performance of tomatoes (Manal et al., 2017). In fact, alkaloids, saponins, flavonoids and tannins present in *Capsicum annuum*, combined with the humic and fulvic acids and other nutrients present in compost tea, justify the effectiveness of the obtained results. All these active molecules enabled this extract to reduce tomato pathologies, ensuring better growth close as possible to the effect of chemical treatment (T5).

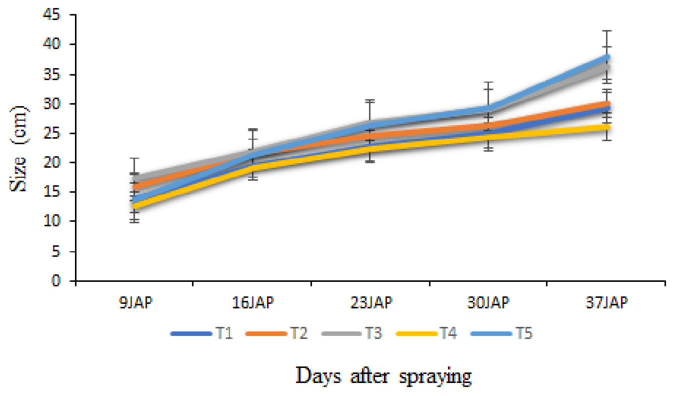

Figure 4 : Variation of plant height over time between treatments

T1 (aqueous chilli extract), T2 (compost tea), T3 (aqueous chilli extract + compost tea), T4 (negative control), T5 (positive control).

3.2.2. Differences in flowers set between treatments over the time after spraying

Flowers are the reproductive structures of flowering plants, which produce nectar and pollen to attract pollinators that ensure fertilization and thus fruit and grain production. In the present study, flower production was favourable to plants from treatments T2 (8 flowers) and T3 (9 flowers), which showed significant differences at 9 (p=0.0114), and 16 DAS and at 16 (p=0.0416) DAS (Figure 5). The decreased number of flowers per plant between treatments was described as T3<T2<T5<T1<T4. at 37 DAP, marking the end of flowering and the beginning of plant senescence. These results are consistent with those who revealed aqueous compost extracts to have the capacity of increasing the number of flowers per plant, justified by the availability of chemical substances (humic acid, fulvic acid and other nutrients contained in compost tea), making compost tea an excellent foliar fertilizer (Mouria et al., 2010).

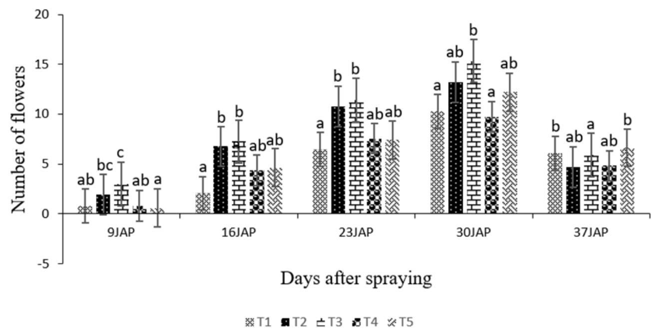

Figure 5 : Variation in mean number of flowers per treatment between treatments as affected by spraying time (DAP)

T1 (aqueous chilli extract), T2 (compost tea), T3 (aqueous chilli extract + compost tea), T4 (negative control), T5 (positive control). Bars bearing the same letters at each day after planting are not significantly different at the indicated level of significance.

3.3 Influence of treatments application on the development of chlorosis

The average number of attacked plant leaves from different bioinsecticide treatments was lower than the ones observed in the control treatment, which showed the highest number (Figure 6). However, the evolution of attacks on tomato leaves varied considerably from one day after spraying to another. Additionally, the combination of compost tea + aqueous chilli extract was more effective than that of other insecticides on tomatoes through its broad spectrum, in line with results who showed that aqueous chilli extract was the bioinsecticide that best stabilised tomato diseases (Diouf et al., 2022). The efficacy of this combination could be attributed to the active molecules it contains (phenolic compounds, alkaloids and flavonoids), as previously pointed out by (Bouchelta et al., 2005). All these active molecules could have enable compost tea + *C. annuum* aqueous extract mixture to significantly reduce the number of infected leaves.

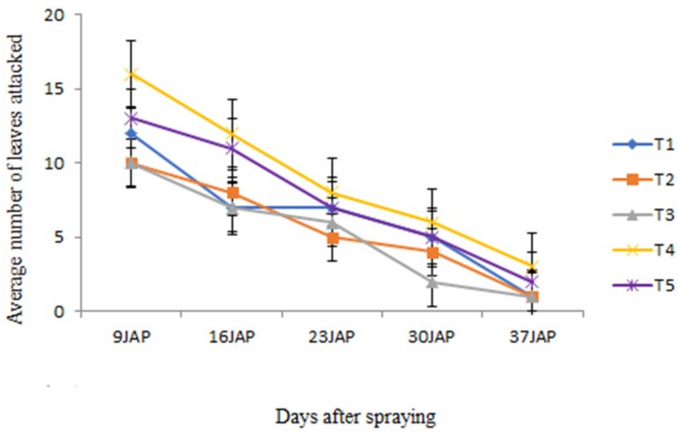

Figure 6 : Evolution of chlorosis after different insecticide treatments

Aqueous chilli extract (T1), compost tea (T2), aqueous chilli extract + compost tea (T3), negative control (T4), positive control (T5).

3.4 Impact of insecticide treatments on the development of anthracnose

Tomato plants treated with insecticide had a significant (p = 0.001) reduction of their fruit rot rate (Figure 7), the control plants indicating higher rate values since they received none of the insecticidal spray. The negative control showed rot disease on fruits at a rate twice higher than that of the combined treatments made up of compost tea + aqueous chilli extracts. The combined treatment compost tea + aqueous chilli extract was more effective in reducing the rot disease on fruits than the other insecticides in the order T3<T1<T2<T4<T5. These results are in agreement with those who reported the combination of deltamethrin+manate 80 to achieve the lowest damages rate on tomatoes compared to the negative control (Adje et al., 2009). In fact, the reduction in the rate of rotting in tomato fruits in field treated bioinsecticides could be the feedback of different biological effects that they inflict to pathogens as causative agents of the disease (Tunaz and Uygun, 2002).

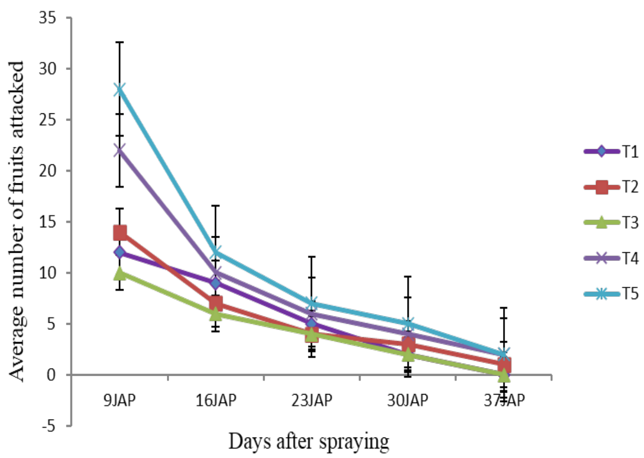

Figure 7 : Variation of fruits attacked by rot disease (Anthracnose symptoms) after different insecticide, applications

Aqueous chilli extract (T1), compost tea (T2), aqueous chilli extract + compost tea (T3), negative control (T4), positive control (T5). Bars bearing the same letters at each day after planting are not significantly different at the indicated level of significance.

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3.5 Effect of treatments on fruits number

The number of tomatoes fruits yielded after organic treatments was greater than that of the chemical insecticide treatment (T4) at 37 days after spraying (Figure 8), characterised by 2 to 2.5 folds increased (p=0.0004) in the number of fruits recorded on treatments (T3 and T5) compared to treatment T4. However, the mean number of fruits from the negative control (7.5) was higher than that of the aqueous chilli extract alone (6.96), in line with the results of Diouf et al. (2022) who recorded low yields and increased pest damage on plots treated primarily with chilli extract pointed out the relative efficiency of aqueous extracts based on *Cistris* and *Inula* alone or in combination, indicating that the efficacy of an aqueous extract depends on the active ingredients it contains and the host plant from which it was prepared (Lokbani, 2018).

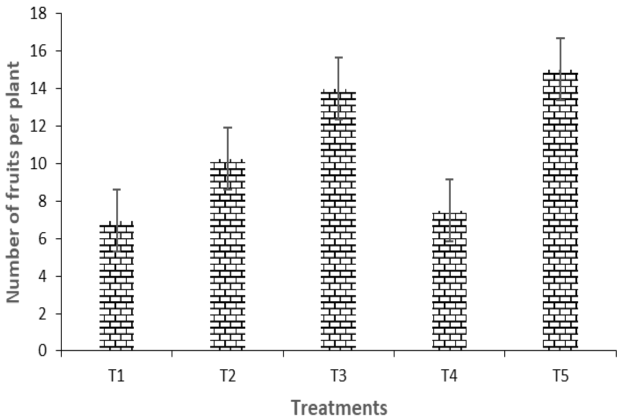

Figure 8 : Differences in tomatoes fruits number yielded by various applied treatments.

Aqueous chilli extract (T1), compost tea (T2), aqueous chilli extract + compost tea (T3), negative control (T4), positive control (T5). Bars bearing the same letters at each day after planting are not significantly different at the indicated level of significance.

3.6 Responses of insecticide application on tomato fruit weight.

The effect of the different treatments on tomato yield is illustrated by Figure 9. The fresh tomatoes weight varied from one treatment to another due to pests attacks and environmental pressure, with yield of 0.37 t/ha, 0.86 t/ha, 1.045 t/ha, 1.46 t/ha and 2.04 t/ha accounting respectively for treatments T4, T2, T1, T3 and T5.

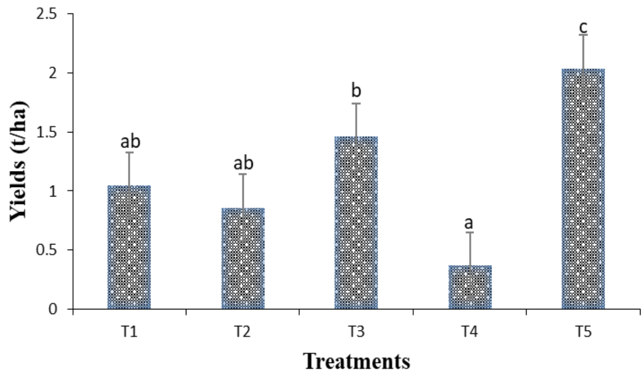

Figure 9 : Variation in tomatoes fruits yielded (t/ha) by various applied treatments.

Aqueous chilli extract (T1), compost tea (T2), aqueous chilli extract + compost tea (T3), negative control (T4), positive control (T5). Bars bearing the same letters at each day after planting are not significantly different at the indicated level of significance.

The highest fruit yields were recorded in treatments T3 (1.46 t/ha) and T5 (2.04 t/ha), that were significantly greater (p=0.0473) than that of other treatments in the order T5 > T3 > T2 > T1 > T4 (confer Figure 9). These results line with those of who reported highly significant differences in tomato fresh weight compared to the negative control in response to application of neem oil extracts (Harouna et al., 2019 ; Ngando et al., 2022). However, the difference with our experiment could be justified by the fact that the active compounds in neem, mainly azadirachtin, have a more broad spectrum of activity compared to that of chilli and compost tea extracts.

3.7 Expression of links between different evaluated parameters on tomato plants

Plant size was moderately correlated (r = 0.45), although not significant (p = 0.08) to the number of flowers per plant (Table 2), indicating that these parameters are not linked each other. Plant size was strongly and significantly correlated with fresh fruits weight (r = 0.75; p = 0.001) and the number of fruits per plant (r = 0.88; p = 0.001), in line with findings, the greater the tomato plant size, the most likely the increased yield (Sawadogo et al., 2021). Moreover, the positive and significant correlation between fruit fresh weight, plant size and the number of fruits formed per plant was in agreement with the reported findings good fruits yield was closely linked to the plant size and its number of fruits set (Samba, 2001 ; Sall et al., 2003 ; Bâ et al., 2014). In the opposite, the higher the number of flowers per plant, the greater number of fruits per plant was obtained (r = 0.71; p = 0.002).

Table 2: Pearson correlation between the different parameters evaluated

Parameters	r/p values	Fresh fruits weight	Fruits per plant	Plant size
Fresh fruits weight	1			
Fruits per plant	r =0.634	1		
	p =0.011			
Plant size	r = 0.750	r = 0.880	1	
	p = 0.001	p = 0.0001		
Flowers per plant	r = 0.152	r = 0.712	r = 0.459	
	p =0.588	p = 0.002	p = 0.084	1

r: Pearson correlation (r = 1: perfect; r > 0.8: very strong; 0.5 ≤ r ≥ 0.8: strong; 0.2 ≤ r ≥ 0.5: average; 0.1 ≤ r ≥ 0.2: weak; r = 0: none); p: probability

4. Conclusion

In the present research, the efficacy of *Capsicum annuum* aqueous extracts and compost tea was investigated in the field on some diseases symptoms that negatively impact the growth and yield of tomato. Results have indicated that the chemical pesticide and the biological treatments based compost tea and *C. annuum* extract were effective in controlling chlorosis and anthracnose of tomatoes, therefore, improved plant size and number of flowers per plant, which contributed to boost yields over the negative control plants. The combined aqueous compost tea and *C. annuum* extract was considered as the best of all the applied insecticides, and hence, could be proposed as a substitute to synthetic pesticides for the control chlorosis and anthracnose of tomatoes, for a sustainable yield improvement of this Solanaceae. These biocides are suggested as an eco-friendly phytosanitary product, with no negative effect on human/animal health ant his environment.

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